Candidate Loci are Revealed by an Initial Genome-wide Association Study of Juvenile Osteochondritis Dissecans

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Background: Osteochondritis dissecans (OCD) is a condition that oftentimes causes significant knee pain in pediatric patient populations. If left untreated, OCD significantly increases the risk of developing degenerative osteoarthritis along with its associated consequences and costs. Although a genetic component has been suggested to play a role in this disorder, few studies have been carried out in order to determine the underlying genetic etiology of this relatively common complex trait. The goal of our study was to perform an initial genome-wide association study (GWAS) to uncover candidate loci associated with the pathogenesis of OCD.

Methods: Blood samples were acquired from 2 cohorts, aged 0 to 18 years old, consisting of 209 OCD cases and 1855 population-matched controls. Agencourt Genfind DNA isolation technology was used to isolate high-quality DNA from each sample. Genotype data was then generated utilizing the Illumina Infinium BeadChip array to examine single-nucleotide polymorphisms (SNPs).

Results: In an initial GWAS analysis of our cohort, where a SNP was excluded if the Hardy-Weinberg Equilibrium test $P < 0.0001$, the minor allele frequency < 5%, and the genotyping call rate < 90%, we obtained our first results for OCD. Although there was no SNP strictly reaching the threshold for genome-wide significance at this early stage, multiple SNPs (35) at several loci revealed evidence of suggestive association with OCD ($P < 5.0 \times 10^{-5}$).

Conclusions: The results from our preliminary study are encouraging. Herein we not only discuss the relevance and applicability of GWAS in studying a genetic basis for OCD, but have also identified top signals that may suggest loci involved in coordinated expression as well as a transcription factor involved in development that may be highly relevant to this trait.

Clinical Relevance: If genetic predispositions for OCD are detected early enough in life, attempts at activity modification, counseling, and orthopaedic monitoring may successfully reduce progression of this condition, which may lead to progressive osteoarthritis in the third to fourth decade in at-risk patients.

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The authors declare no conflicts of interest.

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Key Words: osteochondritis dissecans, OCD, pediatrics, genetics, knee

(*J Pediatr Orthop* 2015;00:000–000)

Osteochondritis dissecans (OCD) is a relatively common cause of knee pain and dysfunction in the pediatric and adolescent patient population. This condition is defined as "A focal idiopathic alteration of subchondral bone with risk for instability and disruption of adjacent articular cartilage that may result in premature osteoarthritis."[1] It initially manifests as a softening of the overlying articular cartilage (Fig. 1) that can progress to early articular cartilage separation, partial detachment of an articular lesion, and eventual osteochondral separation with loose bodies.[2–9] Pediatric patients characteristically report a long history of playing competitive and/or recreational sports without recollection of a specific traumatic incident and typically present with localized knee pain, joint stiffness, and occasional locking attributable to the underlying pathology.[3]

Recent epidemiologic studies on large electronic medical records demonstrate high rates of OCD in patients aged 12 to 19, and lower rates in patients 6 to 11 years old—the former group having 3 times the risk of OCD as the latter.[10] It is very rarely reported in those under age 6. In addition, in this population-based cohort study of juvenile OCD of the knee, male patients had a much greater incidence of OCD and almost 4 times the risk of OCD compared with female patients.[10] Looking at the population as a whole, the incidence of OCD is estimated to be 0.02% to 0.03% based on a survey of knee radiographs.[11]

OCD of the knee is subcategorized into juvenile OCD (JOCD) and adult OCD, based on the status of the distal femoral physis. JOCD has a substantially better prognosis, with > 50% of cases showing healing within 6 to 18 months from nonoperative treatment alone. The remaining patients with JOCD and the vast majority of patients with adult OCD frequently require operative intervention for healing.[8] Without appropriate and timely treatment, JOCD has the potential to result in the development of significant osteoarthritis,[12,13] a physically painful, emotionally draining, and financially burdening potentially preventable sequela.[14]

The etiology of OCD remains unclear and no single theory regarding the cause of OCD is universally accepted. Leading thoughts on the cause of OCD include

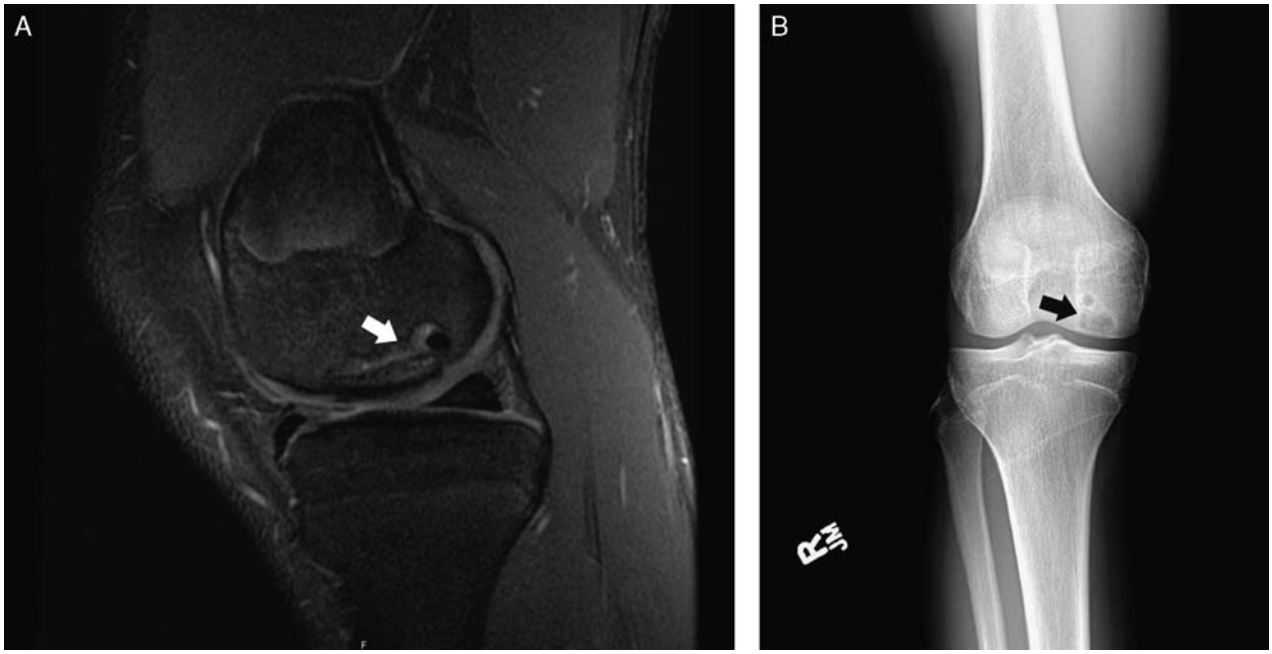

FIGURE 1. Anteroposterior radiograph (B), sagittal proton density magnetic resonance imaging (A) demonstrating osteochondritis dissecans lesion (arrows) of the medial femoral condyle with cystic changes in a 17-year-old boy.

repetitive microtrauma, secondary effects associated with vascular insufficiency, avascular necrosis, inherited factors, and genetic predisposition.[11] In fact, a genetic basis for OCD continues to garner increased support.[15–27] Case studies dating as early as 1925 to 1967 called into question the genetic aspects of OCD.[15] More recently, with increased awareness of the disease process, the literature has reflected an increase in reports of familial forms of OCD. Several cases of monozygotic twins, both with OCD, have been reported over the past few years.[16–21] Of particular interest, a report by Mei-Dan et al[18] on 11-year-old monozygotic twin brothers presenting with OCD describes near-identical symptom onset that was simultaneous. These twin brothers also had a parallel clinical course, suggesting a genetic component to OCD. In a case report of dizygotic twins by Kenniston et al,[20] the brothers presented with OCD of the elbow in which the nondominant side was affected, increasing suspicion for a genetic component rather than a repetitive microtrauma etiology for the disease process.

Lending further support of a genetic basis for OCD, many diseases with an obvious inheritance pattern have been associated with the development of OCD. In a series looking at patients with Stickler syndrome (a genetic collagen disorder), 30% of patients have been shown to present with multiple OCD lesions.[18] OCD lesions have also been reported as occurring in association with Dwarfism,[23–27] Osgood-Schlatter's disease, and Scheuermann's disease.[15]

The identification of pediatric patients at increased risk for OCD lesions based upon their genetic predisposition could allow for earlier intervention to help prevent injury and minimize the risk of the long-term detrimental complications of osteoarthritis. Herein, we sought to perform a small sample preliminary genome-wide association study (GWAS) to detect potential single-nucleotide polymorphisms (SNPs) and genetic loci associated with the pathogenesis of OCD in hopes of elucidating more targeted genetic foci for future research. To our knowledge no prior published sequencing or GWAS effort has been conducted on pediatric patients with OCD lesions.

METHODS

All subjects were recruited from 2006 to 2011 at the Children's Hospital of Philadelphia (CHOP) under a protocol approved by the CHOP Institutional Review Board. The case and control group age distribution was from 0 to 18, with equal sex representation in both groups. Subjects with OCD (cases) were clinically diagnosed by a surgeon within the Division of Orthopedic Surgery before being approached to participate in the study. Control subjects were recruited from within the CHOP Network at routine or well-child visits and did not have any chronic medical conditions based on a standard screening intake questionnaire. The control group was deliberately not filtered in any way in order to have the best population-based control set possible. This is the most conservative approach in which to pursue detection of association, in line with most previous studies from the study team and others in the complex disease arena. Parental informed consent and assent was obtained from each study participant before enrollment and collection of blood samples.

Our study cohort consisted of 209 JOCD cases and 1855 population-matched controls of European ancestry.

Agencourt Genfind DNA isolation technology was used to isolate high-quality DNA from blood samples. DNA samples were analyzed for purity and sufficient molecular weight using a Nanodrop 1000 spectrophotometer and Agilent 2100 Bioanalyzer, respectively.

Genotype data were generated using the Illumina Infinium BeadChip array to examine SNPs. Hardy-Weinberg Equilibrium test[28] was conducted using PEDSTATS. SNPs that failed the Hardy-Weinberg Equilibrium test ($P < 0.0001$) were flagged and excluded. An initial assessment of genotype quality focused on: (i) the proportion of genotype calls for each marker (across all individuals) and (ii) the number of observed discrepancies when a sample is genotyped twice for the same marker. Identifying subjects for whom a large proportion of genotypes are missing helped identify potential problems with sample preparation. An elevated proportion of missing genotypes indicated a poor assay. We excluded samples with low sample call rates (< 90%), low SNP genotype call rate (< 97.5%), and discrepancies in duplicate genotyping. SNPs were also excluded if the minor allele frequency was < 5%.

RESULTS

On the basis of the analysis of the 209 JOCD subjects, we uncovered a number of potential OCD-associated loci. Although there was no SNP crossing the threshold for genome-wide significance at this early stage, 35 SNPs at several loci showed suggestive association with OCD ($P < 5.0 \times 10^{-5}$) (Table 1).

A cluster of identified SNPs (8) on chromosome 13 [$P = 1.56 \times 10^{-6}$, odds ratio (OR) = 1.70] are harbored within a genomic region encoding an antisense RNA (*POU4F1-AS1*). The top signal on chromosome 7 ($P = 1.32 \times 10^{-6}$, OR = 2.11) lies near to a related microRNA (*MIR148A*). As such, the current top signals suggest loci involved in coordinated expression. In addition, the third top SNP, rs1464500 ($P = 3.40 \times 10^{-6}$, OR = 1.65), located on chromosome 12, resides in the gene encoding *SOX5*, the protein product of which is a well-studied transcription factor involved in embryological and cartilage development.

TABLE 1. Results of GWAS of 209 OCD Cases and 1855 Controls of European Ancestry

SNP	CHR	BP	OR	*P*
rs12539793	7	25971860	2.113	1.32E-06
rs9574246	13	79038429	1.699	1.56E-06
rs1464500	12	24389660	1.65	3.40E-06
rs6759076	2	40494866	0.5953	6.04E-06
rs2135507	4	83586671	0.5453	8.70E-06
rs17813162	13	79020968	1.63	8.72E-06
rs717827	2	40497501	0.5834	1.07E-05
rs208390	20	52590227	1.587	1.21E-05
rs7812753	8	75691260	1.83	1.30E-05
rs2186414	11	58530764	1.717	1.48E-05
rs566084	11	58460073	1.682	1.65E-05
rs1008163	4	55054156	0.5502	1.73E-05
rs2834213	21	34792910	1.609	1.84E-05
rs11698483	20	6694203	1.912	2.32E-05
rs6832891	4	55056644	0.5592	2.67E-05
rs263305	2	158080876	2.274	2.71E-05
rs7329914	13	79033325	1.546	2.81E-05
rs1783217	11	58468718	1.698	2.95E-05
rs1043313	9	36214971	0.5683	3.00E-05
rs584799	18	72845315	1.549	3.02E-05
rs10896825	11	58553771	1.647	3.24E-05
rs9487258	6	110263644	0.5701	3.28E-05
rs17503184	2	40400332	0.6267	3.49E-05
rs2759253	1	42021616	0.4301	3.57E-05
rs585702	18	72852959	0.647	3.84E-05
rs9544800	13	79028087	1.534	3.88E-05
rs3822691	5	156809248	1.956	3.91E-05
rs7558770	2	40395982	0.6303	4.15E-05
rs7992108	13	79054390	1.531	4.21E-05
rs9601049	13	79019727	1.53	4.46E-05
rs3824359	9	139105229	1.678	4.56E-05
rs10800778	1	201400572	0.5772	4.70E-05
rs253345	5	150016115	1.525	4.81E-05
rs4326932	13	79025470	1.525	4.90E-05
rs9318562	13	79047083	1.523	4.95E-05

BP indicates base pair; CHR, chromosome; GWAS, Genome-Wide Association Study; OCD, osteochondritis dissecans; OR, odds ratio; SNP, single-nucleotide polymorphism.

DISCUSSION

Although a genetic component has been previously suggested to play a role in OCD, to our knowledge, no prior published sequencing or GWAS effort has been conducted on unrelated pediatric patients with OCD lesions. In a recent systematic review of the literature, Gans et al[29] analyzed 35 articles—34 of which were of a low level of evidence (≤4), with the remaining article identifying one candidate gene by genome-wide linkage analysis within a single large family cohort (*ACAN*).[30] Gans et al[29] concluded that the need for future genetic studies of higher quality is both timely and important to further analyze the genetic nature of OCD.

Our results uncovered a number of potential loci that may be fundamental to OCD pathophysiology. As mentioned above, the cluster of SNPs on chromosome 13 in association with the top signal on chromosome 7 suggest loci involved in coordinated genetic expression. In addition, the SNP located on chromosome 12, rs1464500, resides in the gene encoding *SOX5*. As noted earlier, *SOX5* is a well-studied transcription factor involved in development and may be highly relevant to this trait. Specifically, *SOX5* has been found to work in close conjunction with related transcription factors in targeting chondrocytic genes to influence chondrocyte and subsequent cartilage development[31–33] and has been shown to enhance the function of Sox9 in multiple consecutive phases of chondrocyte maturation.[34,35]

Both the demonstration of coordinated expression as well as the gene-specific locations of the identified SNPs lends credence to the support of a genetic component to JOCD. If genetic predispositions are discovered early enough in life, patients may significantly benefit. For example, if a pediatric or adolescent patient presents to clinic with knee pain but without any signs of ligamentous or meniscal pathology, and the treating physician is aware of

the patient's genetic predisposition to develop OCD, further clinical and radiologic workup may be performed sooner in the diagnostic regimen. Ideally JOCD legions will be discovered in the earlier stages of pathogenesis and appropriate treatment and counseling can be provided in an effort to prevent progression to unstable legions and/or subsequent surgery, and secondary risks of osteoarthritis. Similarly, patients sustaining JOCD of one knee, and subsequently discovered to have a genetic predisposition for the condition, can be more appropriately monitored on their contralateral side. If possible, patients discovered to have the "JOCD genetic variant(s)" early on in life may even be counseled to avoid certain sports while focusing on sports with lower repetitive impact on the lower extremities to prevent the disease process altogether. After all, the relationship between genetic predisposition and environmental factors, including repetitive motion and mechanical loading, remains complex and largely unknown at this stage. This field is still in its infancy but as concepts mature, we can explore such gene-environment interactions, including ascertaining metrics of mechanical loading. Of course, these are hypothetical improvements in patient care at this phase of the research, but they are certainly important to begin considering.

Although these results are encouraging and the potential benefits to patients are substantial, it is crucial to note that this preliminary study is still underpowered to detect genome-wide significance. Despite the aforementioned reassuring findings, top signals observed may well represent "false-positive" results. Larger patient cohorts of cases and controls must be enrolled, and GWAS or whole exome sequencing analysis must be conducted in a multistaged validation effort to truly validate our findings and demonstrate genome-wide significance. However, we present these preliminary findings here to underscore the impressive potential in applying recent technological and computational advances in genome-wide genotyping in search of a genetic basis for this complex disease process. Recent epidemiologic studies have demonstrated racial differences in the incidence of OCD of the knee, with African Americans demonstrating the highest rates, followed by white, Hispanic, and Asian patients, respectively.[10] This may also be a factor to be considered in future genetic studies.

Continued funding and a multicenter collaborative effort will enable continued significant strides in this field. The information gained from such a study would be invaluable for delineating familial predisposition for developing OCD. Consequently, attempts at activity modification, counseling, and orthopaedic monitoring may be successful at reducing injury rates. This could in turn decrease rates of surgery in the young population and decrease these patients' risk of developing osteoarthritis and subsequent comorbidities later in life.

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